

IN THE CLAIMS

Please cancel Claim 3. Please amend Claims 1 and 24 as indicated below.

Claim 1 (currently amended): A method which is prognostic for a preneoplastic/neoplastic disease afflicting a subject vertebrate, wherein said disease affects a tissue, which tissue normally expresses MN/CA IX protein, but loses or has significantly reduced MN/CA IX expression upon carcinogenesis, said method comprising:

(a) detecting MN/CA IX protein or MN/CA IX polypeptide in a sample comprising preneoplastic/neoplastic tissue taken from said vertebrate,

(b) quantitating the level of said MN/CA IX protein or MN/CA IX polypeptide in said sample,

(c) comparing the level of MN/CA IX protein or MN/CA IX polypeptide of step (b) to the average level of MN/CA IX protein or MN/CA IX polypeptide in comparable samples taken from vertebrates afflicted by the same preneoplastic/neoplastic disease as the subject vertebrate, and

(d) determining that said subject vertebrate has a poorer prognosis if the level of MN/CA IX protein or MN/CA IX polypeptide of step (b) is higher than the average level of

MN/CA IX protein or MN/CA IX polypeptide in said comparable samples, than if said MN/CA IX protein or MN/CA IX polypeptide of step (b) were absent or at a significantly reduced level in said sample relative to said average level;

wherein said MN/CA IX protein is encoded by a nucleotide sequence selected from the group consisting of:

- (1) SEQ ID NO: 1's coding region;
- (2) nucleotide sequences that hybridize under stringent hybridization conditions of 50% formamide at 42 degree C. to complement of SEQ ID NO: 1's coding region; and

- (3) nucleotide sequences that differ from SEQ ID NO: 1's coding region or from the nucleotide sequences of (2) in codon sequence due to the degeneracy of the genetic code;

wherein said MN/CA IX protein or said MN/CA IX polypeptide is specifically bound by the M75 monoclonal antibody that is secreted from the hybridoma VU-M75, which was deposited at the American Type Culture Collection under ATCC No. HB 11128; and

wherein said tissue is selected from the group consisting of gastric mucosa, gallbladder, biliary ducts, ductal cells of duodenal glands, testis including ductular efferens and rete testis, ovary including surface coelomic epithelium and rete ovari, basal cells of hair follicles, and central nervous system choroid plexus.

Claim 2 (previously presented): The method of Claim 1 wherein said normal expression of MN/CA IX protein in said tissue indicates that 40% or more of the cells of said tissue express MN/CA IX protein.

Claim 3 (canceled).

Claim 4 (original): The method of claim 1 wherein said vertebrate is a mammal, and wherein said preneoplastic/neoplastic disease is selected from the group consisting of preneoplastic/neoplastic diseases of gastric mucosa, gallbladder, biliary ducts, and ductal cells of duodenal glands.

Claim 5 (original): The method of claim 1 wherein said vertebrate is a human patient, and said preneoplastic/neoplastic disease is selected from the group consisting of neoplastic diseases of gastric mucosa, gallbladder, biliary ducts and ductal cells of duodenal glands.

Claim 6 (original): The method of claim 1 wherein said neoplastic disease is gastric cancer, and wherein said sample is taken from the invasion front of said gastric cancer.

Claim 7 (original): The method of claim 5 wherein said neoplastic disease is a tumor, and said sample is taken from said tumor and/or from a metastatic lesion derived from said tumor.

Claim 8 (previously presented): The method of claim 1, wherein immunohistochemical staining with MN/CA IX-specific antibody is used to detect and quantitate MN/CA IX protein in the sample, and wherein the quantitating step (b) comprises determining an immunoreactivity score of cells in said sample comprising:

(b1) determining the percentage of immunoreactive cells, wherein the percentage of immunoreactive cells is assigned

a value of 0 if no immunoreactive cells,  
a value of 1 if less than 10% immunoreactive cells,  
a value of 2 if between 11% and 50% immunoreactive cells, or

a value of 3 if more than 50% immunoreactive cells;

(b2) determining the intensity of immunostaining of the immunoreactive cells, wherein the intensity of MN/CA IX immunostaining is assigned

a value of 0 for staining equal to a negative control,  
a value of 1 for weak staining,

a value of 2 for moderate staining, or

a value of 3 for strong staining; and

(b3) adding the value for the percentage of immunoreactive cells found in step (b1) and the value for the intensity of immunostaining found in step (b2) to obtain the immunoreactivity score;

wherein the comparing step (c) comprises determining the immunoreactivity scores of said comparable samples analogously to the determination of the immunoreactivity score of the sample from the subject vertebrate in steps b(1) to b(3), and averaging said immunoreactivity scores from said comparable samples; and

wherein if the immunoreactivity score of the subject vertebrate sample determined in steps b(1) to b(3) is above the average immunoreactivity score of said comparable samples, concluding in step (d) that said vertebrate has a poorer prognosis than if said immunoreactivity score is at or below said average immunoreactivity score.

Claim 9 (original): The method of claim 1, wherein a poorer prognosis is measured in terms of shortened survival, increased risk of recurrence of said preneoplastic/neoplastic disease, or in diminished or refractory response to treatment.

Claim 10 (original): The method of claim 1, wherein said disease is neoplastic and comprises a tumor, or a tumor and one or more metastatic lesions derived from the tumor, and wherein a poorer prognosis is measured in terms of shortened survival, increased risk of recurrence of said neoplastic disease, or diminished or refractory response to treatment, following treatment and/or surgical removal of the tumor, or the tumor and said one or more metastatic lesions.

Claim 11 (original): The method of claim 1, wherein said preneoplastic/neoplastic sample is a formalin-fixed, paraffin-embedded tissue sample or a frozen tissue sample.

Claim 12 (canceled).

Claim 13 (canceled).

Claim 14 (previously presented): The method according to claim 1, wherein said detecting step (a) comprises the use of an assay selected from the group consisting of Western blots, enzyme-linked immunosorbent assays, radioimmunoassays, competition immunoassays, dual antibody sandwich assays, immunohistochemical staining assays, agglutination assays, fluorescent immunoassays cytofluorometry.

Claim 15 (canceled).

Claim 16 (original): The method according to claim 1, wherein said detecting step (a) comprises the use of the monoclonal antibody secreted by the hybridoma VU-M75 which has Accession No. ATCC HB 11128.

Claim 17 (canceled).

Claim 18 (previously presented): The method according to claim 1, wherein the detecting step (a) is by immunohistochemical staining, and wherein the quantitating step (b) comprises determining the percentage and/or the intensity of immunostaining of immunoreactive cells.

Claim 19 (original): The method of claim 1, wherein said vertebrate is a mammal.

Claim 20 (original): The method of claim 19, wherein said mammal is a human.

Claim 21 (previously presented): The method of claim 1, wherein said prognostic method is used as an aid in the

selection of treatment for said preneoplastic/neoplastic disease afflicting said vertebrate, further comprising concluding that said patient is a high risk patient in need of adjuvant therapies, if said determination in step (d) indicates a poorer prognosis.

Claim 22 (original): The method of claim 1 wherein said sample is taken from the invasion front of said preneoplastic/neoplastic disease, and said comparable samples are analogous invasion front samples.

Claim 23 (original): The method of claim 22 wherein said preneoplastic/neoplastic disease is a neoplastic disease.

Claim 24 (currently amended): A method which is prognostic for a preneoplastic/neoplastic disease afflicting a subject vertebrate, wherein said disease affects a tissue in which 40% or more of the cells normally express MN/CA IX protein, but said tissue loses MN/CA IX expression or expression of MN/CA IX is significantly reduced upon carcinogenesis, said method comprising:

(a) taking a tissue sample from the invasion front of said preneoplastic/neoplastic disease;



(b) determining whether MN/CA IX protein or MN/CA IX polypeptide is absent or at a significantly reduced level in said invasion front sample as compared to the level that said MN/CA IX protein or MN/CA IX polypeptide is normally expressed in said tissue, when said tissue is unaffected by said disease; and

(c) concluding that if said MN/CA IX protein or MN/CA IX polypeptide is neither absent nor at such a significantly reduced level in said invasion front sample, that the subject vertebrate has a poorer prognosis than if said MN/CA IX protein or MN/CA IX polypeptide were absent or at a such a significantly reduced level in said invasion front sample; and

wherein said tissue is selected from the group consisting of gastric mucosa, gallbladder, biliary ducts, ductal cells of duodenal glands, testis including ductular efferens and rete testis, ovary including surface coelomic epithelium and rete ovarii, basal cells of hair follicles, and central nervous system choroid plexus.